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### **4,6-alpha-Glucanotransferase activity occurs more widespread in Lactobacillus strains and constitutes a separate GH70 subfamily**

Leemhuis, Hans; Dijkman, Willem P.; Dobruchowska, Justyna M.; Pijning, Tjaard; Grijpstra, Pieter; Kralj, Slavko; Kamerling, Johannes P.; Dijkhuizen, Lubbert; Gerwig, Gerrit J.

*Published in:*  
Applied Microbiology and Biotechnology

*DOI:*  
[10.1007/s00253-012-3943-1](https://doi.org/10.1007/s00253-012-3943-1)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2013

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Leemhuis, H., Dijkman, W. P., Dobruchowska, J. M., Pijning, T., Grijpstra, P., Kralj, S., Kamerling, J. P., Dijkhuizen, L., & Gerwig, G. J. (2013). 4,6-alpha-Glucanotransferase activity occurs more widespread in Lactobacillus strains and constitutes a separate GH70 subfamily. *Applied Microbiology and Biotechnology*, 97(1), 181-193. <https://doi.org/10.1007/s00253-012-3943-1>

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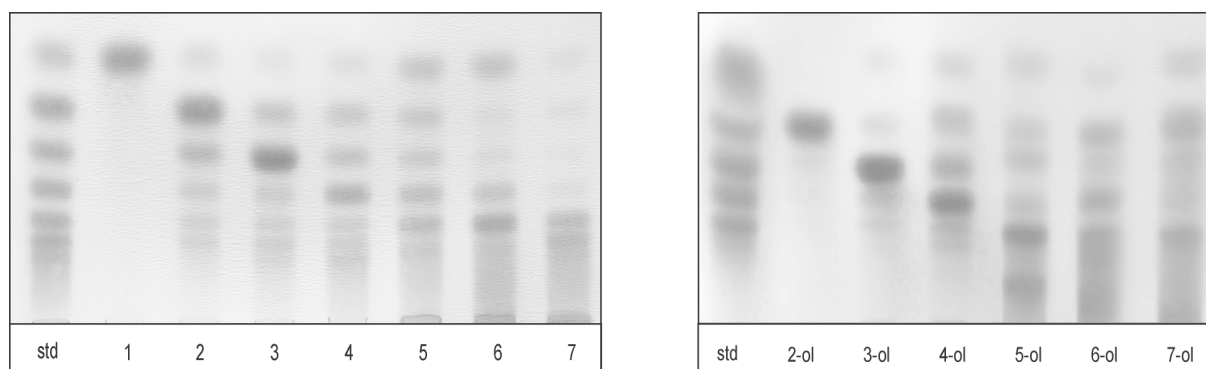
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## Supplemental data

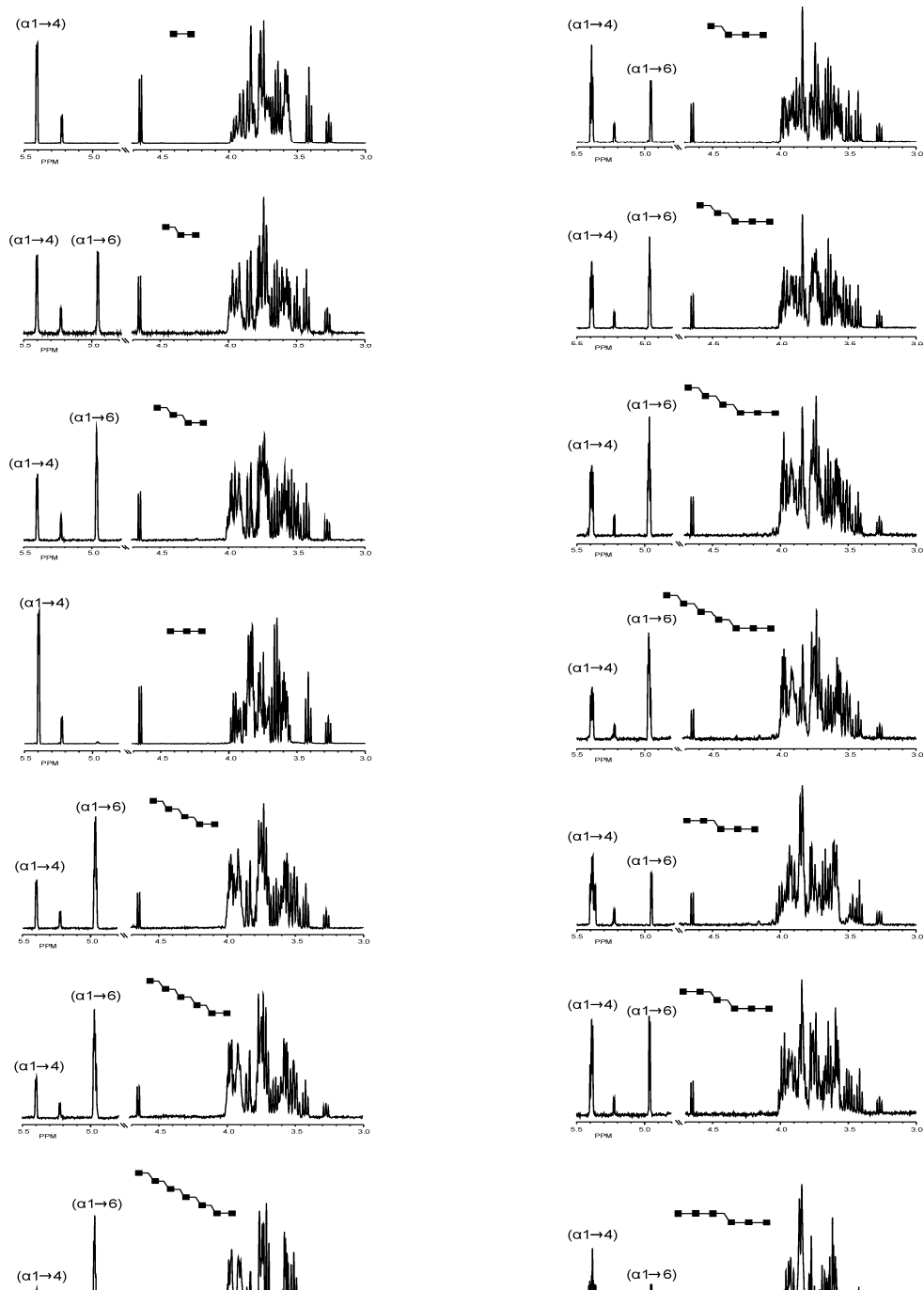
### Structural characterization of linear isomalto/malto-oligomer products synthesized by the novel GTFB 4,6- $\alpha$ -glucanotransferase enzyme from *Lactobacillus reuteri* 121

Justyna M. Dobruchowska, Gerrit J. Gerwig, Slavko Kralj, Pieter Grijpstra, Hans Leemhuis, Lubbert Dijkhuizen, and Johannes P. Kamerling

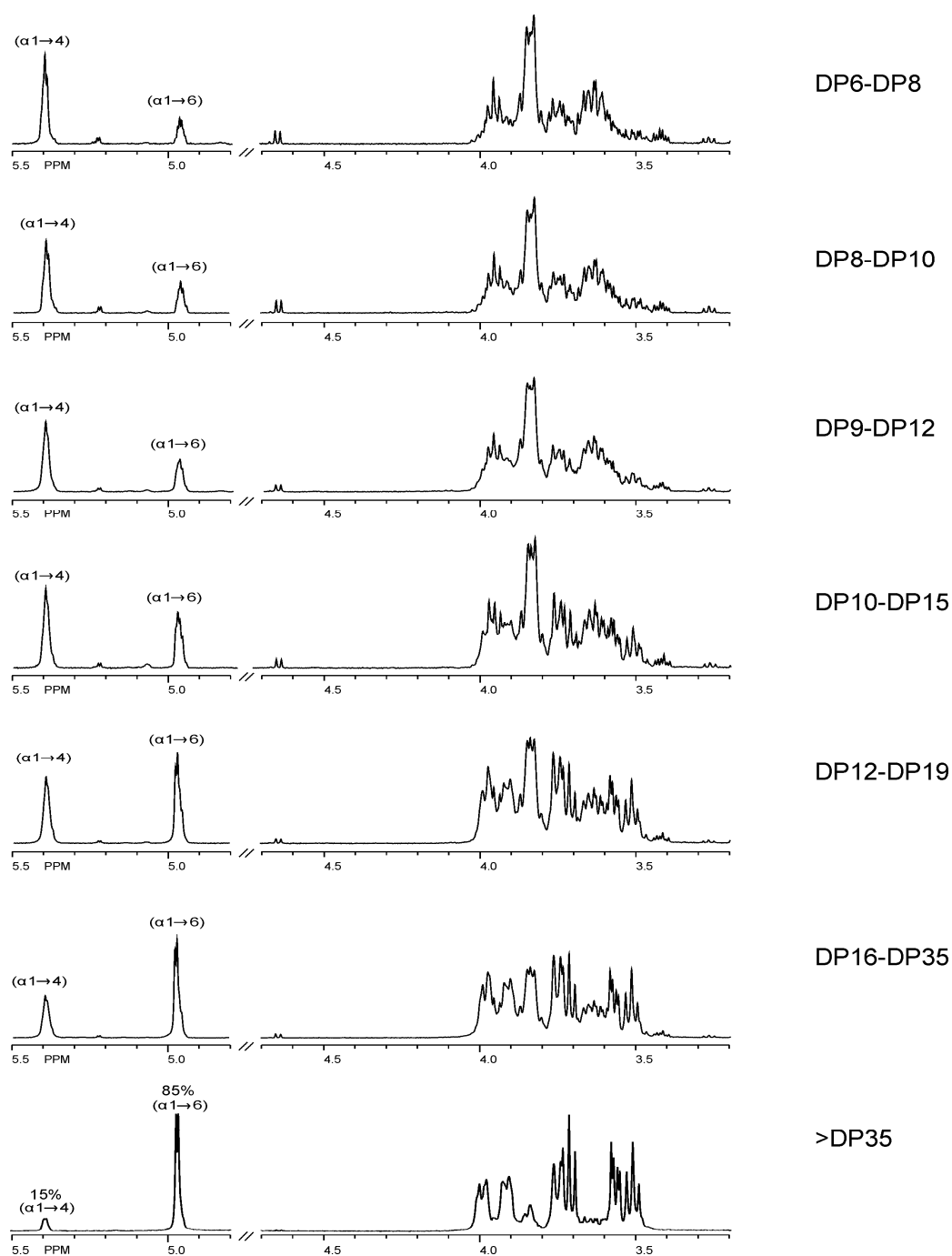
Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands



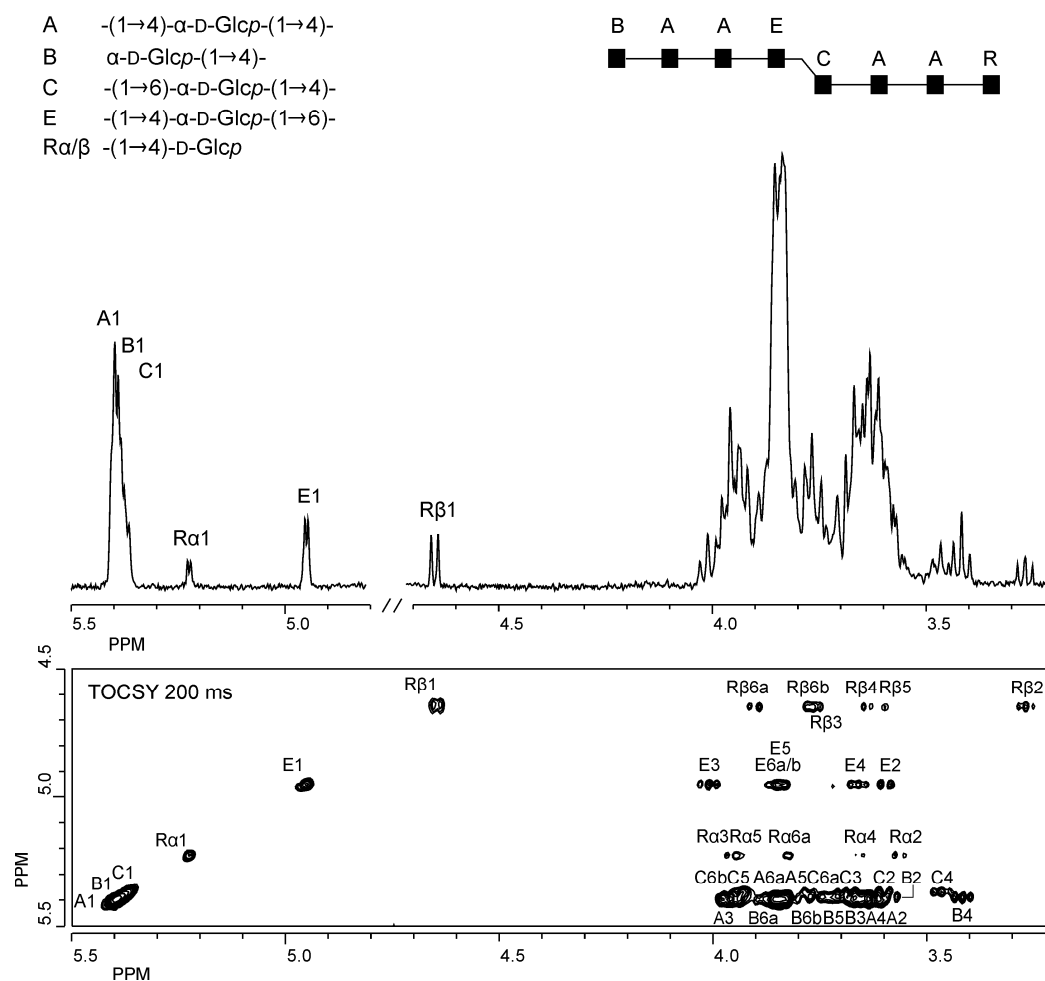
**Fig. S1.** TLC showing the GTFB activity on different malto-oligosaccharides(-alditols). [DP1, DP2(-ol), DP3(-ol), DP4(-ol), DP5(-ol), DP6(-ol), DP7(-ol)]. Incubations of 100 mM oligosaccharide(-alditol) solutions were carried out with 500 nM GTFB for 72 h at 37°C and pH 4.7.



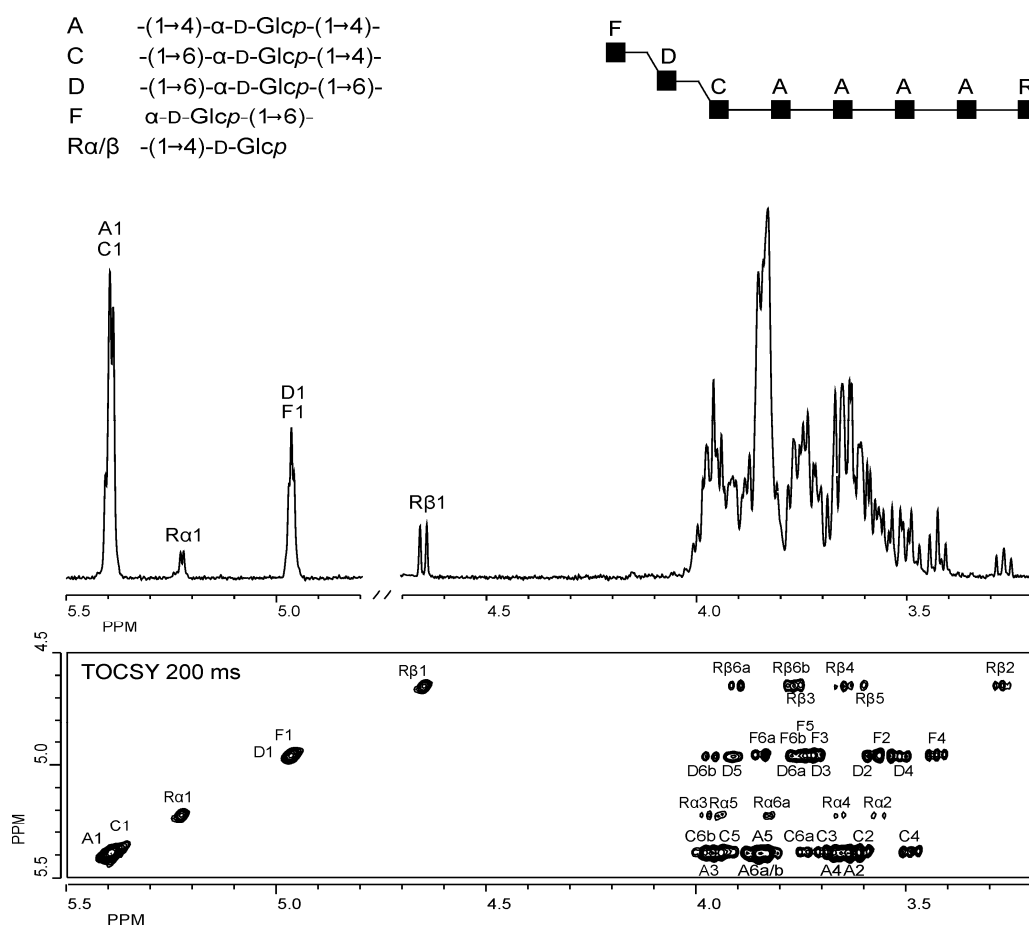
**Fig. S2.**  $^1\text{H}$  NMR spectra of the products found in the generated oligosaccharide mixtures after the incubation of maltose and/or maltotriose with GTFB for 72 h at 37°C and pH 4.7. Note that the reducing Glc units occur as  $\alpha/\beta$  mixture.



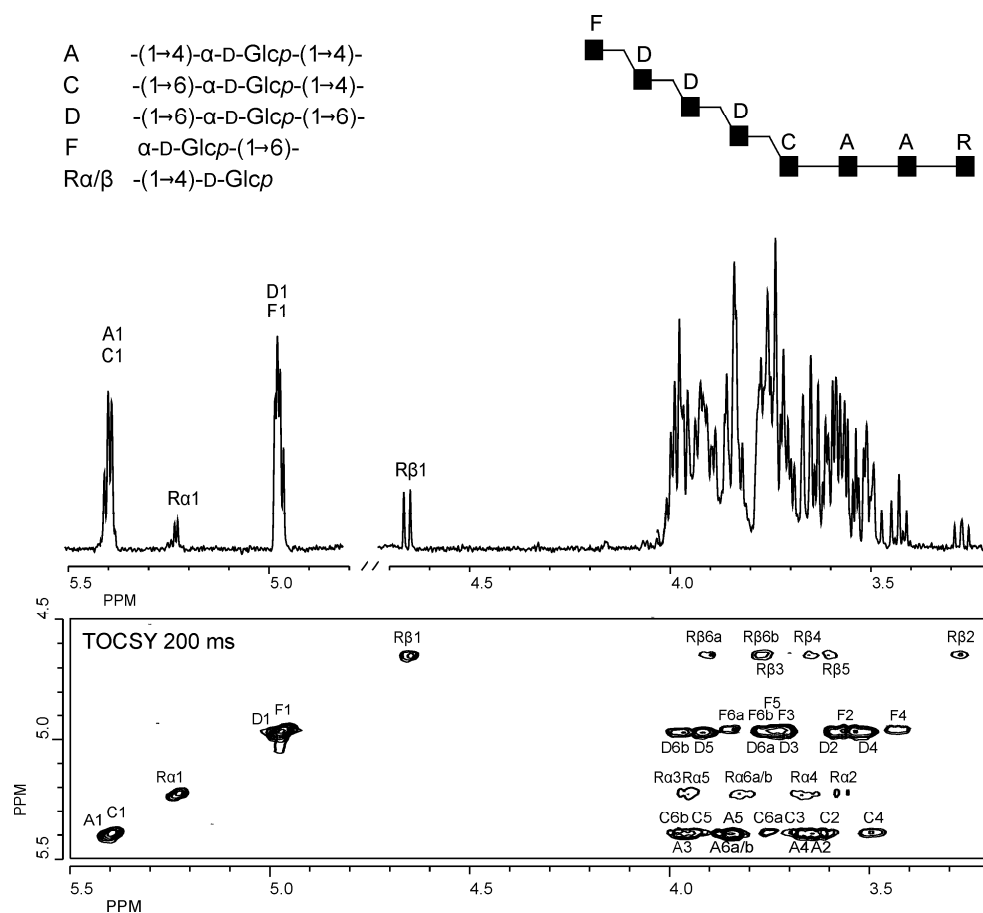
**Fig. S3.** One-dimensional  $^1\text{H}$  NMR spectra of different Bio-Gel P-2 fractions with increasing DP (DP6–DP8, DP8–DP10, DP9–DP12, DP10–DP15, DP12–DP19, DP16–DP35, DP>35), obtained from the incubation of maltoheptaose (DP7) with GTFB, showing that the amount of  $(\alpha 1 \rightarrow 6)$  linkages increases with increasing DP.



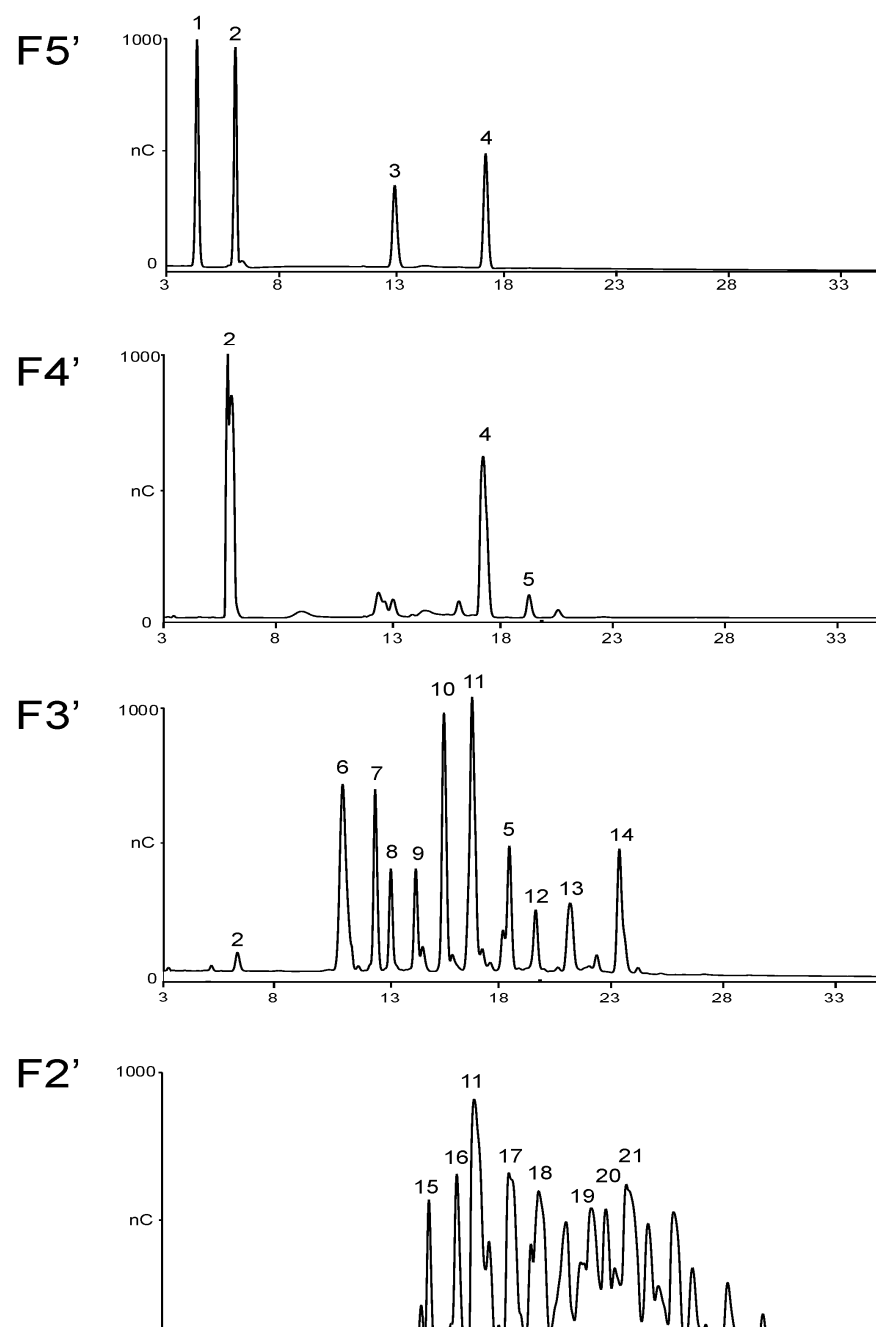
**Fig. S4.** One-dimensional  $^1\text{H}$  NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide  $[\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-D-Glcp}]$ .



**Fig. S5.** One-dimensional  $^1\text{H}$  NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide  $[\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-D-Glcp}]$ . Note the similarity of the TOCSY spectrum with those in Figure 5 and S6.



**Fig. S6.** One-dimensional  $^1\text{H}$  NMR and TOCSY (200 ms) spectra of a DP8 product oligosaccharide  $[\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}-(1\rightarrow4)\text{-D-Glcp}]$ .



**Fig. S7.** HPAEC-PAD subfractionation (0 to 500 mM NaOAc gradient in 100 mM NaOH) of Bio-Gel P-2 fractions **F2'**-**F5'** on CarboPac PA-1 [incubation of maltopentaitol (DP5-ol) with GTFB].